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FREDRICK J. STARE, PH.D., M.D., Professor of Nutrition, Schools of Medicine and Public Health, Harvard University; Associate in Medicine, Peter Bent Brigham Hospital, Boston. Editorial Office, 695 Huntington Ave., Boston, Mass.

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NUTRITION REVIEWS

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NUTRITION AND FOOD TECHNOLOGY

The past decade has witnessed a number of distinct developments in the processing of foods, some of which have already attained acceptance by both industry and consumer. Others await the critical tests that will decide their future practical value. Although the methods, equipment, and products resulting from these developments are diversified, in every instance there has been a common goal, namely, to produce for the American table final products that have improved flavor, color, texture, and nutritive value. An added objective in some instances has been to minimize or eliminate, in so far as possible, the time and the effort of kitchen labor previously required in preparation.

Perhaps, in the experience of the consumers, the most popular products currently resulting from such developments have been the citrus and other fruit or vegetable concentrates, many but not all of which are produced in frozen form. Few food products have gained consumer acceptance so rapidly and widely.

In general, such products have shown improvements in flavor, color, and nutritive value. To accomplish this regularly and uniformly is neither simple nor easy from the standpoint of the food processor. It has necessitated the most rigid quality control from both a chemical and a microbiologic basis. In a number of producing plants, vitamin C assays are conducted on an hourly schedule during all the operating periods, both on the raw materials and on the finished products, for the vitamin C content is one of the most practical yardsticks of efficient plant procedures. Quantitative microbiologic analyses are also invaluable means of guarding the quality of the final product, assuring the proper choice of raw materials, and maintaining strict sanitary control of equipment in the processes employed.

These precautions require extra personnel and expense, but the continued success and popularity of the new products are dependent on strict maintenance of such quality control. If and when any one of the rapidly growing list of producers of such products lowers the safeguards of quality, the result may be serious to the entire industry and possibly disastrous to the individual company.

Many food manufacturing companies do not assign top priority to nutritive value in their list of product requirements, but this factor is seldom omitted from the list and is usually not far behind eye-appeal, flavor desirability, and convenience. The growing consciousness of nutritive value on the part of consumers is responsible for part of the emphasis on this factor, but to the food industry itself must be given a large part of the credit for constant improvements in their processes and components to make their products more adequate from a nutritional standpoint.

Among the developments that illustrate this trend are: (a) improvements in heat exchange which permit more rapid heating and subsequent cooling of both liquid and solid products during food processing, (b) devising of mechanical methods that accelerate heat penetration in hermetically sealed containers, and (c) the design and operation of a new system that will make it possible to fill food containers at much higher temperatures than was previously possible. Each of these developments is directed toward a reduction in the extended time at high temperature otherwise required for safely processing certain foods, and the

net result in each successful case will be more appetizing and more nutritious food products.

Recently a method of processing foods that includes addition of small quantities of antibiotics has been suggested as a means of reducing the more conventional time and extent of heat processing, but this method, like some of the others, is still in the laboratory or experimental stage. Much further work is necessary before commercial application of the use of antibiotics is feasible.

Electronic means of accomplishing the same objective have been subject to much recent research. It would seem that high-frequency radio applications may have some limited usefulness in this respect, especially from the standpoint of blanching foods.

Another field of current interest is the application of cathode rays, or electrons, by which means foods may be sterilized in the practical absence of heat, for in this instance the temperature of foods is raised only a few degrees with the radiation exposure necessary.

Such procedures require evaluation from many standpoints before they can be considered appropriate for commercial food application, as they sometimes cause undesirable changes in color and flavor. The enzymes in the plant and the animal tissues that constitute foods have been found to be much more resistant to cathode ray irradiation than are the microorganisms that normally accompany them, thereby requiring special consideration. The effects of such irradiation on fats, carbohydrates, and proteins, as well as on other nutritive components of foods have been subjects of much study in the postwar years. To date, such irradiation appears to cause losses in nutritive value of a magnitude generally comparable to (though no more severe than) the losses resulting from the more common processes now used.

Which, if any, of these processes will survive the pilot-plant stage and the actual commercial trials that may follow would be difficult to predict. The American public, however, may be assured that from the vast amount of technologic and nutritional research now going on the net results will be better foods and better nutrition of our people and the people of the world in the years to come.

That our many food industries, educational institutions, and food research laboratories have directed their food developmental projects toward attaining these net results indicates a trend toward better foods for better nutrition through food technology, a trend that one may confidently expect to become even more apparent in the future.

Bernard E. Proctor
Department of Food Technology
Massachusetts Institute of Technology
Cambridge, Massachusetts

ADAPTATION TO UNDERNUTRITION

The physiologic adjustments which result from a decrease in nutrient intake are sometimes spoken of as "adaptation to undernutrition." H. H. Mitchell (*J. Am. Dietet. Assn.* **20,** *511 (1944)*) emphasized the ability of the animal organism to undergo physiologic adjustment to various levels of nutrient intake and stated as a principle the theorem: "If an animal in equilibrium with its food supply (meaning a well-nourished animal) is subjected to nutritional stress, such as an inadequate (or an excessive) supply of one or more of the essential nutrients, the animal will react in such a way as to minimize, as far as possible, or to undo entirely the effects of the nutritional stress." Although some may hold that such physiologic changes do not necessarily constitute true adaptation, there is no doubt that some alterations occur in response to changes in nutrient

intakes. Studies and definition of these changes are of considerable practical importance.

The Minnesota experiment on semistarvation has permitted a quantitative description of the adaptation to caloric restriction (H. L. Taylor and A. Keys, *Science* **112,** *215 (1950)*). The human subjects who underwent six months of dietary restriction which resulted in the loss of some 24 per cent of their body weight and the development of signs of starvation, made the following physiologic changes which resulted in lowering of caloric requirements: (1) A decrease in the caloric expenditure for physical activity which decrease accounted for two-thirds of the total caloric saving. The voluntary curtailment of activity amounted to 60 per cent of this decrease while reduced energy cost of performance of a given task in direct proportion to loss of body weight was responsible for the other 40 per cent. (2) The metabolic rate fell 31 per cent, of which approximately two-thirds was due to shrinking of the metabolizing tissue mass while one-third would seem to be attributable to the decreased intensity of metabolism per unit of active tissue. (3) The work done by the heart decreased by 50 per cent. These phenomena, and other observed phenomena, demonstrate that man can call into play adaptative mechanisms which permit him to meet the situation of caloric restriction.

E. Wertheimer and V. Ben-Tor (*Brit. J. Nutrition* **4,** *1 (1950)*) have investigated the adaptation of carbohydrate metabolism in the rat to underfeeding. Under certain conditions these workers found that partial undernutrition results in an increase in liver and muscle glycogen and an intensive deposition (synthesis?) of glycogen in some adipose tissues. Their experiments were made as follows: A group of 3-month-old rats weighing 105 to 120 g. was fed a stock diet (carbohydrate 70 per cent, casein 20 per cent, fat 10 per cent) at one-half the usual daily level. A 12 per cent loss of body weight resulted. A slightly younger (to result in the same terminal weights) control group was offered the identical diet ad libitum. Both groups were fasted overnight, then given 5 g. of the diet, killed at intervals of from six to forty-eight hours, and the glycogen of liver, muscle, and adipose tissue determined by the method of C. A. Good, H. Kramer, and M. Somogyi (*J. Biol. Chem.* **100,** *485 (1933)*). Samples of brown interscapular fat, mesenteric fat, and mixed fatty tissue from the groin, perirenal, and genital sites were analyzed.

At all intervals from six to twenty-four hours after the test feeding the liver-glycogen concentration was significantly higher for the chronically underfed rats. Glycogen content of muscle was higher in the same group until nine hours after the test meal.

The glycogen content of the adipose tissues of the underfed group six hours after feeding was 2.67, 0.46, and 0.16 per cent for brown interscapular fat, mesenteric fat, and mixed fat, respectively. This is in contrast to values of 0.66, 0.05, and 0.00 for the same areas in the well-fed animals. Although absorption was slightly delayed in the undernourished group, the workers did not feel that this accounted for the observed differences. Furthermore, upon the basis of adrenalin and insulin lability of the body stores of glycogen, they opined that there was no remarkable difference in glycogen stability to account for the observations. These results indicate that the chronically underfed rat shows a temporary increase in glycogen storage when food is metabolized. This increased deposition of glycogen is especially striking in the adipose tissues.

Although these workers do not speculate upon the finding, it may be of importance to recall the at least superficial similarity of this observation and some aspects of Selye's "general-adaptation-syndrome" (H. Selye, "Textbook of Endocrinology," *p. 837, Université de Montréal, Montreal, Canada (1947)*). This syndrome is the sum of systemic reactions which follow the long-continued exposure of the organism to a

stress condition. It is characterized by enlargement of the adrenal cortex with increased secretion of corticoid hormones, involution of lymphatic organs, development of gastrointestinal ulcers, and various metabolic changes. The initial application of stress produces the so-called "alarm reaction" with phenomena representing signs of damage as well as inauguration of defenses, such as adrenal stimulation. If stress be continued, the "stage of resistance" may occur during which the increased resistance to the stress agent results in adaptation. Finally, very prolonged exposure to the stress may lead to failure to maintain the adaptation with the occurrence of the "stage of exhaustion."

Carbohydrate metabolism is profoundly altered during the general-adaptation-syndrome. Hyperglycemia (increased blood sugar) with depletion of liver glycogen is observed during the alarm reaction. This, presumably, is mediated by release of adrenalin. In some instances (anoxia) there may occur an increase in liver glycogen, perhaps due to the increased secretion of adrenal cortical hormones. It is tempting to raise the question whether this mechanism accounts for the increased liver (and other) glycogen under the stress of starvation. Certainly studies of undernutrition must be made with awareness of the general-adaptation-syndrome.

The findings of Wertheimer and Ben-Tor (*loc. cit.*) of increased glycogen in adipose tissue during starvation deserve comment. Adipose tissue has not been widely recognized as containing glycogen. E. Tuerkischer and E. Wertheimer (*J. Physiol.* **100,** *385 (1942)*) have studied the polysaccharide which occurs in adipose tissue and concluded that it is glycogen. They found that it increased considerably in concentration for one to three days following realimentation of a fasting rat. The interscapular (brown) fat was especially active in depositing glycogen in such animals, the content reaching as high as 2.2 per cent within six hours after feeding. The brown fat was empty of glycogen, however, within four to five days after realimentation. It was implied that this glycogen was deposited as a precursor for fat synthesis.

In view of the possible relationship of the adaptation to undernutrition to the more general adaptation syndrome it is of some interest that adrenalectomy decreases greatly the glycogen accumulation in both liver and brown fat of realimented animals. The response is restored toward normal by administration of concentrates of adrenal cortical hormones. It is of course well known that the 17-hydroxy corticoids enhance glycogen concentration.

Studies designed to test the speculations advanced in this review should be informative.

VITAMIN K AND BLOOD COAGULATION

Recent papers by Henrik Dam, the discoverer of vitamin K, and his collaborators of the Polytechnic Institute of Copenhagen, Denmark, give a new and interesting light on the mechanism of coagulation of blood, the role of vitamin K, and the effect of Dicumarol. Until 1943 it was generally accepted that prothrombin was a unitary principle. The low clotting power of blood in vitamin K deficiency, as well as in Dicumarol poisoning was due to the fall in prothrombin content of the blood. Then, with insufficient prothrombin, and even in the presence of an abundant supply of thromboplastin and calcium ions, thrombin was formed in insufficient amounts to insure proper conversion of fibrinogen into fibrin. In 1943, however, A. J. Quick (*Am. J. Physiol.* **140,** *212 (1943)*) indicated that prothrombin might be a complex of two

substances: component A, labile, which disappeared from stored plasma after prolonged storage; and component B, stable, which constituted the "body of the prothrombin complex."

Between 1943 and 1947 several papers (e.g., P. Nolf, *Arch. internat. de pharmacodyn. et de therap.* **70,** *5 (1945)*; R. Feissly, *J. Suisse de Med.* **32,** *696 (1945)*) suggested that prothrombin might be a complex of even more than two components. In 1947 A. J. Quick (*Am. J. Physiol.* **151,** *63 (1947)*) deduced from careful studies of the anomalies of coagulation of the blood of members of two families afflicted with congenital hypoprothrombinemia that three factors might intervene, besides thromboplastin and calcium, in the formation of thrombin. The reaction would be represented by:

Prothrombin (A and B) + labile factor + thromboplastin + calcium = thrombin. Thus, the blood of one of the families studied was deficient in component B. A similar deficiency is caused by administration of Dicumarol. In the second family the hypoprothrombinemia was due to lack of a second factor, designated as component A. Quick indicated that this principle might be the one to decrease in vitamin K deficiency. Finally, a labile factor which disappears from stored plasma due to slow oxidation might also be essential for prothrombin activity.

However, in 1948 H. Dam and E. Søndergaard showed that the latter theory did not adequately represent the phenomena observed in the clotting of normal blood, Dicumarol blood, and vitamin K-deficient blood. First, Dam (*Nature* **161,** *1010 (1948)*) observed that mixtures of Dicumarol and vitamin K-deficient plasmas which, according to Quick's scheme should have presented a normal clotting time, in fact did not. Secondly, Dam and Søndergaard (*Biochim. et biophys. acta* **2,** *409 (1948)*), pursuing the analysis, showed that in mixtures of two plasmas of the same kind (normal-normal, vitamin K deficient-vitamin K deficient, Dicumarol-Dicumarol) or in mixtures of normal plasma with either Dicumarol or vitamin K-deficient plasma, the clotting time never declined below that of the most rapidly clotting plasma. On the other hand, mixtures of Dicumarol plasma and vitamin K-deficient plasma showed a decrease in clotting time to a point below that of the most rapidly clotting component but above normal in all cases. This was interpreted as signifying that both Dicumarol plasma and vitamin K-deficient plasma are primarily lacking in one and the same component, in addition to which Dicumarol plasma is lacking in a second component and vitamin K-deficient plasma in a third.

Dam and his collaborators then started investigating the nature of the factor in the plasma of vitamin K-deficient chicks which accelerates the coagulation of plasma from chicks treated with Dicumarol and of the factor in Dicumarol plasma which accelerates the coagulation of plasma from vitamin K-deficient chicks. The results of this important and painstaking work have appeared in two papers (Ø. Sørbye, I. Kruse, and H. Dam, *Acta chem. Scandinav.* **4,** *549, 831. (1950)*).

Using adsorption and elution procedures, a small protein fraction was isolated from the plasma of vitamin K-deficient chicks which decreased the prothrombin time of Dicumarol plasma. This factor, called the kappa factor, presents the additional following properties: the addition of larger amounts of this factor over and beyond the small amounts needed to shorten the prothrombin time of Dicumarol plasma has no effect. Its addition to plasma from vitamin K-deficient chicks does not alter the prothrombin time. It has no effect over stored plasma (unless supplied with small amounts of fresh plasma, containing the most labile factor, called V factor).

Similarly, the same workers were able to concentrate another factor, which they called delta factor, present in Dicumarol

plasma, which accelerates the coagulation of plasma from vitamin K-deficient chicks. This factor, again, was active in small amounts, addition of greater quantities having no further influence on the clotting time of vitamin K-deficient plasma. Conversely, it had no effect on the prothrombin time of Dicumarol plasma nor of fresh normal chicken plasma. Addition of highly active preparations of factor V had no accelerating effect on the prothrombin time of the vitamin K-deficient plasma used in this series of experiments.

Thus, at least four factors are needed in addition to thromboplastin and calcium to form thrombin: prothrombin proper, the kappa factor, the delta factor, and the V factor. Vitamin K deficiency causes specifically only a deficiency in the delta factor, while other causes, Dicumarol poisoning, for example, may produce deficiencies in other factors. Many points remain to be cleared, and nutritionists, physiologists, and clinicians will be anxious for further developments of this problem.

EFFECT OF COCOA UPON HUMAN CALCIUM AND NITROGEN RETENTION

Earlier reviews (*Nutrition Reviews* **2,** *68* (*1944*); **4,** *251* (*1946*)) referred to experiments in which large amounts of cocoa had been fed to white rats during the course of measurement of calcium and phosphorus retention. In the interpretation of such experiments, it is important to keep in mind that ingestion of many types of common foodstuffs tends to decrease the efficiency of calcium absorption. Hence, an intake of cocoa, in amounts far above those ingested in human practice, would have little bearing upon the common practice of drinking cocoa or eating foodstuffs containing cocoa.

A later investigation (H. H. Mitchell and T. S. Hamilton, *J. Nutrition* **31,** *377* (*1946*)) with various types of cocoa indicated that the effect of the cocoa upon calcium retention varied greatly with the brand of cocoa used but, in all experiments at the above level of cocoa ingestion, the experimental rats retained less calcium than their controls. However, a preliminary report of studies with human subjects in whom the cocoa intake was appreciably lower than in the rodent studies suggested that at the levels used there was no deleterious effect on calcium utilization (Mitchell and J. M. Smith, *J. Am. Med. Assn.* **129,** *871* (*1945*)).

In view of the wide interest in the effect of cocoa on the utilization of calcium, particularly that in milk, M. L. Bricker, J. M. Smith, T. S. Hamilton, and H. H. Mitchell (*J. Nutrition* **39,** *445* (*1949*)) conducted an investigation with 8 college women as subjects, over an eight-month term which was divided into eleven dietary periods. The basal, low calcium diet supplied an average of 225 mg. of calcium and 70 to 80 g. of protein per day. Other than in calcium, the basal diet was believed to be adequate. Whole homogenized milk and cocoa powder were added in varying quantities. The cocoa used was described as "a moderate-cost American process cocoa" and had a fat content of 20.22 per cent and an energy value of 5.48 calories per gram. As the amounts of these ingredients were varied, adjustments were made in the intake of other foods in an attempt to maintain a constant ratio of total calories, protein, and fat. The individual caloric requirements of the subjects were supplied by bread and sugar supplements to the basal diet as needed. No restriction in physical activity was imposed throughout the experiment. Distilled water was allowed as desired.

The eleven diet periods consisted of the basal ration with the following alterations: (1) no additions; (2) milk 400 g.; (3) cocoa 5.6 g., milk 400 g.; (4) no additions; (5)

cocoa 58.2 g., milk 420 g.; (6) milk 420 g.; (7) cocoa 28.5 g., milk 420 g.; (8) milk 240 g., vanilla ice cream 185 g.; (9) cocoa 34.8 g., milk 240 g., chocolate ice cream 185 g.; (10) no additions; (11) cocoa 21.0 g. Thus in all, five levels of cocoa were tested, varying from 5.6 to 56.2 g. per day, four in diets containing milk as the major source of calcium, and the fifth in a milk-free diet with a low calcium level. The daily calcium intake provided by these diets varied from 206 to 755 mg.

Aliquot portions of food were selected for analyses at the time of preparation. All foods were analyzed in four-day pools. Fecal and urinary collections likewise were composited into four-day samples. Analyses for calcium, nitrogen, dry matter, and gross energy were made by acceptable procedures.

Probably the most striking result of the entire experiment was the extreme individual variation in calcium retention among subjects and between periods for the same subject. In this regard, the data were strikingly similar to those of F. R. Steggerda and H. H. Mitchell (*J. Nutrition* **31,** *407 (1946)*). No consistent change in calcium balance was noted upon inclusion of cocoa during any of the periods. For all periods and subjects, the daily calcium balances averaged -12.85 ± 11.0 mg. and -8.70 ± 9.7 mg. for the noncocoa and cocoa diets, respectively. This difference was found not to be statistically significant.

When the data were expressed in terms of the calculated degree of calcium utilization, the average utilization for all subjects in all periods on noncocoa diets was 21.3 per cent of the calcium ingested; the same value for all periods and subjects on cocoa diets was 20.0 per cent. Again, the difference was insignificant statistically. The average calcium requirement for all subjects in all periods was 686 ± 52 mg. on cocoa diets and 669 ± 42 mg. on noncocoa diets. Again, because of the high standard deviation, this difference was not significant.

Studies on the urinary and fecal calcium excretion indicated that there was a significant difference in the route of calcium elimination during ingestion of cocoa. With inclusion of cocoa, in twenty-five of twenty-seven comparisons, the urinary calcium levels decreased, and in twenty-three of twenty-seven comparisons the fecal calcium increased above that of adjacent periods during which cocoa was omitted. With cocoa feeding, increases in the fecal excretion of dry matter and nitrogen were observed in each of four dietary comparisons. These increases were accompanied by decreases in urinary nitrogen. The increased fecal nitrogen excretion indicates that there was a lower over-all digestibility of dietary protein in the presence of cocoa. However, the increase in fecal nitrogen was equal to about 84 per cent of the extra nitrogen ingested in the cocoa itself.

Feeding of cocoa at the higher levels was accompanied by more frequent and bulkier stools. The total gross energy value of the feces was observed to increase during feeding of cocoa in each of three possible comparisons. The increase in energy value was in direct proportion to the amount of fecal dry matter excreted. The question was raised by the authors as to whether this increase in gross energy could be accounted for by the presence of undigested cocoa.

In only one of the eleven periods did any abnormal subjective symptoms occur. When 56.2 g. of cocoa were fed per day, headaches, nausea, and decreased appetites were reported, accompanied by frequent voluminous well-formed stools. The severity of these indications resulted in the cessation of this high intake of cocoa ingestion after three four-day test periods instead of the customary five or more four-day tests.

From the data presented in this paper, it would appear that the tolerance of the subjects for cocoa was about 1 oz. per day for the grade of cocoa used. Any possible deleterious effect of cocoa on calcium utilization was completely obscured by variations in

the experimental data within groups and within subjects.

In contrast to the rodent studies, it is noted that the 1 oz. level of cocoa which could be tolerated by adult human subjects was far below the level of 16 per cent of the diet provided to rats (W. S. Mueller and M. R. Cooney, *Nutrition Reviews* **2,** *68 (1944)*; **4,** *251 (1946)*). In terms of body weight, these rats consumed about 1.3 g. of cocoa per day for each 100 g. of body weight, which would be equivalent to about 600 g. per day for a human being weighing 100 pounds. According to the data on the 1948 production and use of cocoa in the United States, the average daily consumption was in the neighborhood of 3 g. Since cocoa is a strong flavoring material, even the ounce level apparently safely consumed by these human subjects represents a large intake, incorporation of which into the diet probably tested the skill of the dietitian responsible for planning and preparing experimental rations which were not completely distasteful to the subjects.

Evidence from other experimental studies likewise suggests that ingestion of moderate amounts of cocoa did not have any demonstrable deleterious effects. G. W. Newell and C. A. Elvehjem (*Science* **99,** *411 (1944)*) observed that young rats grew normally when fed a commercially prepared chocolate milk containing 1 per cent cocoa. In addition, G. Hadary, H. H. Sommer, and J. E. Gonce (*J. Dairy Sci.* **26,** *259 (1943)*) observed, in the course of a roentgenologic study with children, that emptying of the stomach after test meals of chocolate milk drinks was comparable to emptying times with other forms of milk.

MAN'S CALORIE REQUIREMENTS

Early nutritional research was largely concerned with food as the fuel supplying the energy needs of the body. Interestingly enough, the question of total energy requirements seems to have arisen first in connection with animal feeding. Sarrus, a physician, and Rameaux, a physicist at the University of Strasbourg, formulated the "surface law" (caloric expenditure proportionate to the body surface) in the thirties of the last century in the course of researches concerned with the practical problem of estimating food allowances for draft horses used in the mines. In Germany, Voit at Munich and Rubner in Berlin, and in this country, Atwater and Benedict were prominently involved in the matter of energy requirements of man (*see* G. Lusk, "Nutrition," *Hoeber, New York (1933)*).

In the twentieth century, except for the interlude of the two world wars, during which time the importance of calories and the consequences of caloric undernutrition and starvation were pointed out, nutritional research was directed to specific nutrients—vitamins, minerals, and amino acids (*see* C. A. Elvehjem, *Science* **109,** *354 (1949)*). In the days of abundant food supply and in countries with food surpluses, the matter of calorie requirements does not have much appeal. In such instances overweight is likely to be more of a problem than caloric deficit. However, this is definitely not the general picture the world over.

The changes in world consumption of calories and proteins were studied, on the basis of best available data on food supplies for human consumption of approximately 75 per cent of the world's population, by C. Chatfield, M. L. Scott, and J. Mayer (*Milbank Memorial Fund Quart.* **28,** *103 (1950)*). Two facts stand out. First, maldistribution of available supplies with reference to large regions of the world has become more acute than in the preceding decades; this trend is likely to continue. Secondly, the average net available food supply per caput has decreased. Whereas there have been rises in the aggregate supplies of calories, total protein, vegetable

protein, animal protein, and milk protein ranging from 0.7 per cent to 6.4 per cent during 1938 to 1948, the population, despite the war, rose by an estimated 8.3 per cent, thus outrunning, in the true Malthusian way, the increased food supplies. This fact should serve as a stimulus for a realistic re-evaluation of calorie requirements.

The existing tables of calorie requirements are inadequate in two respects: (1) the absolute levels are subject to uncertainty which, when multiplied by the world population, seriously affects agricultural planning; (2) the tables have failed to provide proper mechanics for adjusting the calorie requirements with reference to factors such as age and body size.

The uncertainty as to the absolute levels of caloric intake is illustrated by the fact that the 1948 revision of the daily calorie allowances recommended by the National Research Council included several changes (*National Research Council, Reprint and Circular Series* **129** (*1948*)). The amounts allowed to sedentary men were revised downward, from 2500 to 2400 calories, for sedentary women from 2100 to 2000 calories, for moderately active women from 2500 to 2400 calories. The 1943 and 1945 editions of the Recommended Dietary Allowances gave 3000 calories for "moderately active" men and 4500 calories for "very active" men. The 1948 revision retained the caloric values but upgraded the degree of activity to "physically active" and "doing heavy work," respectively.

These downward revisions are in harmony with the experience of life insurance companies and the data provided by the dietary surveys. Overweight, resulting from prolonged surplus intake of calories, has been shown to be associated with increased mortality. Moderate caloric reductions below the accepted "requirements," such as were instituted in Switzerland during the last war, were associated with a favorable health record (A. Fleisch, "Ernährungsprobleme in Mangelzeiten," *B. Schwalbe, Basel* (*1947*)). The experience in Great Britain was similar. Several nutrition surveys of groups of individuals who were not clinically underfed showed lower intakes than the recommended intake levels (*see* A. Keys, *Ann. Rev. Biochem.* **18,** *487* (*1949*)).

Failure to take into account factors, such as body size, which modify the caloric needs leads easily to erroneous conclusions. This may be illustrated by a nutrition survey of Otomi Indians (R. K. Anderson, J. Calvo, G. Serrano, and G. C. Payne, *Am. J. Pub. Health* **36,** *883* (*1946*); *Nutrition Reviews* **4,** *347* (*1946*)). The estimated average daily intake was 2454 calories. This would appear to be a grossly inadequate intake if compared with the time-honored figure of 3000 calories. However, if it is taken into account that the value of 3000 calories was adopted by the National Research Council for physically active men weighing 70 kg., whereas the Otomi Indians averaged only 56.6 kg., and the caloric allowance is adjusted with reference to the difference in body size, the caloric "deficit" is no longer present.

It was the pressing need for a system of requirements, applicable in different parts of the world and incorporating the effect of such factors as body size, which prompted the Food and Agriculture Organization of the United Nations to establish an International Committee on Calorie Requirements. The report of this Committee ("Calorie Requirements," *FAO Nutritional Studies, No. 5, June 1950*) presents the general working principles as well as specific recommendations for estimating the caloric needs of populations varying in body size, age composition, and environmental temperature. The recommendations are designed for application to groups, not to individuals.

The Committee considered a variety of approaches which could be used for attacking the problem. It chose to base its recommendations on a well-defined reference standard and to extrapolate, by proper quantitative adjustments, to peoples differing from the reference in three major characteristics. The age of the reference man

and the reference woman is 25 years, weight 65 and 55 kg., respectively. They live in the temperate zone, with a mean external annual temperature of 10°C. (50°F.). They are in good health and their nutriture is satisfactory. Their height has not been stated but their body build is characterized as "normal." They consume an adequate balanced diet and are neither gaining nor losing weight. Their activities differ; on working days the man is engaged for eight hours in such activities as working in light industry, driving a truck, or market gardening; the woman is engaged in general household duties, including the care of small children, or in light industrial work. They both spend at least two hours out of doors each day. The daily caloric intake, averaged for a whole year, was set at 3200 and 2300 calories, respectively. The "reference" children are regarded as their younger siblings.

Calorie requirements (E) for adults similar in activity, age, and environmental temperature but differing in weight (W) are computed according to the equation $E = a(W)^{0.73}$ where a = 152.0 for men and 123.4 for women. The caloric values calculated from this equation are close (within 10 calories over a range of 50 to 80 kg. for men, 40 to 70 kg. for women) to those obtained from an equation yielding the total energy expenditure as the sum of resting energy, energy expenditure related to ingestion of food ("specific dynamic action"), and energy expenditure involved in physical activity. It was noted that the method of calculating energy requirements adjusted to body size is applicable only to well-nourished persons of ordinary physical build.

Populations in different parts of the world differ markedly in their age composition, from young populations with high birth and death rates and a short life duration to populations with low birth rates and long life expectancy. Consequently, the factor of age has to be considered in calculating the average per caput per day calorie needs of a population. In older adults the energy expenditure decreases, in part because of the reduction of the basal metabolic expenditure, and in part because of a tendency to reduce the level of physical activity, particularly the nonoccupational activity. The over-all decrease in calorie requirements with increasing age has been estimated, for every ten years beyond the age of 25, as 7.5 per cent of the requirement at 25 years. Thus requirements at the age of 45 years are 15 per cent less than at the reference age of 25 years.

The data on the effects of climate on calorie requirements are not adequate for a refined analysis. Also, variations in the duration of exposure to the outdoor environment and in the quality of clothing modify markedly the influence of climate on the total caloric needs. The most systematic information was obtained for the North American soldiers stationed from several months to two years in various parts of the world. The different groups retained the food habits acquired at home and were engaged in much the same military duties. In these circumstances an inverse relationship was observed between the average voluntary calorie intake and environmental temperature. The Committee recommended that the temperature effect be taken into account by decreasing the allowance by 5 per cent for every 10°C. of mean annual external temperature above the reference temperature (10°C.) and increased by 5 per cent for every 10°C. below the reference temperature. This correction is to be applied to all age levels except nursing infants.

For pregnancy no adjustment appeared necessary during the first two trimesters. For the third trimester a supplement of 450 calories above the nonpregnant requirement has been recommended. The allowance for lactation is 1000 calories per day. This corresponds to energy consumed in producing about 850 ml. of milk per day. The average lactation period is estimated at six months.

For infants from 6 to 12 months of age a daily intake of 110 calories per kilogram of body weight is recommended, in accordance with the suggestions of the National Research Council. The Council's recommended allowances were retained also for children from 1 to 16 years; no adjustments for body size are applied to this age group but the correction for environmental temperature is applied. It was felt that adjustment of the calorie allowances of growing children according to their actual size would be undesirable as the undersized children may need more calories than the well-grown child to attain a normal adult size. Calories for the adolescent group (age 16 to 19 years) should allow for continued growth. The requirements of the males were set at 120 per cent, of the females at 105 per cent, of the calories required by young adults (25 years old) in the given population.

Thus far no reference was made to the adjustment of the caloric intake to activity. Obviously, physical activity does affect the caloric needs. In the National Research Council recommendations the range, according to activity, is from 2400 to 4500 calories for men and from 2000 to 3000 for women. However, in any large population the number of individuals with extreme intakes will be relatively infrequent and the additional calories needed by the more active individuals will be balanced out by the lesser needs of the less active individuals. The Committee suggested that for practical purposes the average activity (and its caloric equivalent) of adult populations may be considered as coinciding with the value for the reference adults. Where exceptional distribution of occupations is present, assessment of activity must be incorporated in the calculation of the average caloric expenditure.

Information about the average per caput calorie requirement of a particular population is needed by the public health authorities as well as the departments of agriculture. The former are concerned with assessment of the adequacy of national caloric intake from the medical point of view, the latter with working out national production and consumption policies aimed at an adequate caloric intake. The FAO calorie requirements, based on the best available information and a considered judgment of an international committee of experts, represents an important step in a more realistic and more accurate assessment of the nutritional needs of populations with widely divergent characteristics.

Nevertheless, the values are presented by the Committee as approximate and tentative. They will be modified, perhaps, as knowledge of the relationship between diet and health is widened and deepened. Medical-statistical research indicates that "ideal" weights of adults do not coincide with the arithmetic averages for a given age group. Knowledge about the effects of the rate of growth on man's life span is nonexistent; animal experiments suggest that the slowing down of the rate of growth may be beneficial. There are no adequate studies on productivity and caloric intake.

Two points in the working philosophy of the Committee deserve acclaim. First, in setting up the calorie requirements the Committee proceeded from the relatively known to the relatively unknown. The reference man and the reference woman, young, healthy specimens of mankind, living in the temperate zone, have been studied more carefully with respect to the anthropometric characteristics, physical fitness, energy expenditure, and food consumption than any other human group. Secondly, the premises on which the system of interpolation is based were stated clearly and expressed in numerical values. Thus, they can be subjected to quantitative tests.

The Committee expressed the hope that the report will not only serve as a practical yardstick but will also stimulate research which, in turn, will lead to a more accurate assessment of calorie requirements. In par-

ticular, there is need for further controlled studies on the quantitative effect of each factor influencing calorie requirements. The existing literature has been summarized by A. Keys (*Nutrition Abstr. Rev.* **19,** *1* (*1949–50*); *J. Am. Med. Assn.* **142,** *333* (*1950*)).

THE CITRIC ACID ENIGMA

Citric acid, the common tricarboxylic acid of citrus fruits, was implicated as an important metabolic intermediate in animal tissues by H. A. Krebs (*Advances in Enzymol.* **3,** *191* (*1943*)). Working chiefly with pigeon breast muscle, Krebs and his colleagues showed that 6-carbon citric acid and 5-carbon alpha-ketoglutaric acid had effects upon tissue metabolism similar to the 4-carbon dicarboxylic acids fumaric, succinic, malic, and oxalacetic which had been previously studied by B. Gözsy and A. Szent-Györgyi (*Z. f. physiol. Chem.* **224,** *1* (*1934*)). Addition of these acids to suspensions of pigeon breast muscle resulted in marked stimulation of oxygen consumption and in the presence of pyruvate, of enhanced disappearance of pyruvate.

In the presence of relatively high concentrations of oxalacetic and pyruvic acids (0.1 M), citrate and alpha-ketoglutarate were shown to accumulate. These observations led Krebs to postulate a mechanism for the oxidation of pyruvate which is now well known as the Krebs tricarboxylic acid or citric acid cycle. In brief, Krebs stated that pyruvate or a product of pyruvate combined with oxalacetate to yield citrate which was then oxidized enzymatically through a series of intermediates which included alpha-ketoglutarate, succinate, fumarate, and malate to oxalacetate. The regenerated oxalacetate was then available for further condensations with pyruvate. In the steady state, this reaction sequence was visualized as continuing in a cyclic manner. This hypothesis was generally well received at the time of its initial promulgation and in the course of the intervening years only a few of many modifications suggested by various workers have withstood further investigation, as well as the initial hypothesis.

The most critical comment about the Krebs citric acid cycle has centered about the intermediate formation of citric acid itself. C. A. Baumann and F. J. Stare (*J. Biol. Chem.* **133,** *183* (*1940*)) reported that citrate had a much less pronounced effect upon the respiration of pigeon breast muscle than the other members of the cycle, and Stare, M. A. Lipton, and J. M. Goldinger (*Ibid.* **141,** *981* (*1941*)) showed that pyruvate utilization was more than twice as great as citrate utilization in this tissue under comparable conditions. These observations led this group of workers to conclude that citric acid was probably not in the main channel of the tricarboxylic acid cycle but represented a side reaction.

A similar view was adopted by H. G. Wood, C. H. Werkman, A. Hemingway, and A. O. Nier (*J. Biol. Chem.* **139,** *483* (*1941*)) and E. A. Evans and L. Slotin (*Ibid.* **141,** *439* (*1941*)) on the basis of their independent classic experiments on carbon dioxide fixation in pigeon liver. In these experiments isotopic carbon dioxide was incubated with liver, oxidizing pyruvate in the presence of malonate, and the alpha-ketoglutarate formed (presumably via oxalacetate and "citrate") was isolated. Upon degradation of the alpha-ketoglutarate, however, the isotope appeared solely in the carboxyl adjacent to the carbonyl group and was not randomized between the two carboxyls as might be expected on the basis of a symmetrical intermediate such as citrate. In addition, Evans and Slotin (*loc. cit.*) showed that addition of nonisotopic citrate to pigeon liver under these conditions did not dilute the radioactivity

of the alpha-ketoglutarate or increase its yield. At this time these data appeared to eliminate citric acid as a true intermediate in the tricarboxylic acid cycle. Wood (*Physiol. Rev.* **26,** *198 (1946)*) suggested that an asymmetric 6-carbon acid such as isocitric or *cis*-aconitic was in all probability the product of the initial condensation reaction of the cycle.

These arguments seemed impregnable until A. G. Ogston (*Nature* **162,** *963 (1948)*) at Oxford proposed a revolutionary concept. Ogston suggested that the synthesis of optically active (asymmetric) compounds from symmetrical precursors in animal tissues could be explained if a three-point contact between an asymmetric enzyme and the symmetrical substrate was postulated. Under these conditions it would be possible for the enzyme to distinguish between two identical groups of the precursor and act selectively on one of them. Since the enzyme aconitase which converts citric acid to *cis*-aconitic acid catalyzes a reaction of this type, Ogston reasoned that the arguments based on isotope distribution (*cited above*) for the elimination of citric acid from the tricarboxylic acid cycle of Krebs might not be valid.

Shortly after this suggestion was made, V. R. Potter and C. Heidelberger (*Nature* **164,** *180 (1949)*) confirmed Ogston's hypothesis in an ingenious way. $C^{14}O_2$ was added to rat liver homogenate oxidizing oxalacetate and pyruvate under conditions that led to citrate accumulation. The C^{14}-tagged citrate was then isolated by silica gel partition chromatography, characterized as a pure compound, and then reincubated with another rat liver homogenate under conditions which led to accumulation of alpha-ketoglutarate. This latter compound was then purified as the 2,4-dinitrophenylhydrazone and the distribution of C^{14} in its carboxyls determined by permanganate oxidation. All of the activity was found to reside in the carboxyl group adjacent to the carbonyl group which agreed with the findings of Wood (*loc. cit.*) and Evans and Slotin (*loc. cit.*). The intermediate isolation of citrate, however, made it clear that the radioactivity derived from $C^{14}O_2$ in the first incubation was contained in only one of the two primary carboxyls of citrate and that liver aconitase had been able to distinguish between these two carboxyls in the second incubation which led to formation of alpha-ketoglutarate labeled in only its alpha-carboxyl.

V. Lorber, M. F. Utter, H. Rudney, and M. Cook (*J. Biol. Chem.* **185,** *689 (1950)*) have reported similar evidence in support of the Ogston concept. In their experiments, oxalacetic acid labeled biosynthetically with C^{14} in its beta-carboxyl (by $C^{14}O_2$ fixation) was converted to citrate by a pigeon liver enzyme. The citrate was then rigorously purified and incubated with another pigeon liver enzyme preparation from which alpha-ketoglutarate was isolated. Degradation of this alpha-ketoglutarate revealed all of the activity to reside in the alpha-carboxyl group in agreement with the findings of Potter and Heidelberger (*loc. cit.*). It appears, therefore, that the asymmetric distribution of isotope in alpha-ketoglutarate synthesized via the Krebs cycle from $C^{14}O_2$ and pyruvate does not constitute grounds for eliminating citric acid as the primary condensation product.

Data on the mechanism of synthesis of citrate in pigeon liver extracts reported by J. R. Stern and S. Ochoa (*J. Biol. Chem.* **179,** *491 (1949)*) also support the view that citric acid is the product of the initial condensation of the cycle. These workers found that synthesis of citrate from acetate and oxalacetate in an ammonium sulfate-fractionated enzyme extract from pigeon liver was dependent upon the presence of coenzyme A (*Nutrition Reviews* **7,** *348 (1949)*), ATP, and magnesium ions. Since the enzyme preparation used in this study was practically devoid of aconitase activity, it was concluded that citric acid rather than

isocitric or *cis*-aconitic acid was the primary condensation product.

J. R. Stern, B. Shapiro, and S. Ochoa (*Nature* **166,** *403* (*1950*)) have reported the crystallization of the enzyme which catalyzes the condensation of active acetate and oxalacetate to yield citrate. This "condensing enzyme" was obtained from pig heart by extraction with phosphate buffer, adsorption and elution from calcium phosphate gel, precipitation with ethanol at −15°C., and fractional precipitation with ammonium sulfate. In the presence of this crystalline enzyme, which was three hundred and thirty times more active than the initial extract of pig heart, citrate was rapidly formed from acetyl phosphate, oxalacetate, magnesium ions, cysteine, and *Escherichia coli* factor. This latter *E. coli* factor, described by E. R. Stadtman (*Fed. Proc.* **9,** *233* (*1950*)) as a transacetylase, allowed substitution of acetyl phosphate for acetate ion plus ATP, used in the previous study. Transacetylase also requires coenzyme A for activity and probably catalyzes the following reaction:

$$\text{Acetyl-PO}_4 + \text{coenzyme A} \xrightarrow{\text{transacetylase}} \text{Acetyl-coenzyme A} + \text{H}_3\text{PO}_4$$

Acetyl-coenzyme A complex appears then to be the immediate source of the C_2 fragment in citrate synthesis. Tests of the reversibility of the citrate-forming reaction carried out by incubating radioactive citrate with condensing enzyme, coenzyme A, and oxalacetate indicated that only small amounts of radioactivity appeared in the oxalacetate when the oxalacetate concentration was low (2 mm. per liter) and practically none when the oxalacetate concentration was high (40 mm. per liter).

Similar experiments by Lorber *et al.* (*loc. cit.*) with pigeon liver extract indicated no radioactivity to be present in oxalacetate if radioactive citrate and coenzyme A were incubated with oxalacetate at 40 mm. per liter. These data appear to indicate that the equilibrium constant for the citrate-condensing reaction markedly favors citrate formation.

The role of coenzyme A in citrate formation in pigeon liver extracts has been confirmed by G. D. Novelli and F. Lipmann (*J. Biol. Chem.* **182,** *213* (*1950*)).

That an acetyl-coenzyme A complex is the immediate source of C_2 fragments for citrate synthesis is indicated by another paper from Ochoa's laboratory. S. Korkes, J. R. Stern, I. C. Gunsalus, and S. Ochoa (*Nature* **166,** *439* (*1950*)) have reported that extracts of *Streptococcus faecalis* which oxidize pyruvate to acetyl phosphate in the presence of inorganic phosphate, coenzyme A, a yeast factor, cocarboxylase, and a hydrogen acceptor, would form citrate in the complete absence of inorganic phosphate providing oxalacetate were present. The conclusion drawn from these experiments is that oxidation of pyruvate to active acetate and its condensation with coenzyme A do not involve inorganic phosphate. Acetyl-coenzyme A complex is then available for decomposition to acetyl phosphate in the presence of inorganic phosphate and Stadtman's transacetylase (*see above*) or to citrate in the presence of oxalacetate and Ochoa's condensing enzyme.

Earlier studies of the role of coenzyme A in pyruvate metabolism in animal tissues by R. E. Olson, E. G. Hirsch, H. Richards, and F. J. Stare (*Arch. Biochem.* **22,** *480* (*1949*)) indicated that citrate formation from pyruvate was markedly affected by coenzyme A levels. In homogenates of heart muscle from pantothenic acid-deficient ducklings, it was found that citrate formation from pyruvate and fumarate was markedly reduced and could be restored by addition of purified preparations of coenzyme A. These data are all in support of the view that decarboxylation of pyruvate occurs prior to, rather than subsequent to, the condensation with oxalacetate and renders

somewhat untenable earlier impressions that a 7-carbon intermediate occurred in the oxidation of pyruvate (Wood, *loc. cit.*; D. E. Green, W. F. Loomis, and V. H. Auerbach, *J. Biol. Chem.* **172,** *389 (1948)*; C. Martius, *Z. f. physiol. Chem.* **279,** *96 (1943)*).

Although many of the enigmatic aspects of the citric acid cycle have been clarified, failure of most tissues to utilize *added* citrate at rates comparable to pyruvate or other intermediates of the cycle, and failure of added nonisotopic citrate to dilute endogenous radioactive alpha-ketoglutaric acid formed from $C^{14}O_2$ pose still unsolved problems. Further studies of metabolic competitions involving citrate and of the thermodynamics of citrate activation might prove interesting.

CARBOHYDRATE UTILIZATION IN YOUNG CALVES

Synthetic milk has been used successfully in raising young calves (A. C. Wiese, B. C. Johnson, H. H. Mitchell, and W. B. Nevens, *J. Dairy Sci.* **30,** *87 (1947)*). In these early studies glucose was used as the carbohydrate component. Recently, workers at the Michigan Agricultural Experiment Station (R. J. Flipse, C. F. Huffman, H. D. Webster, and C. W. Duncan, *Ibid.* **33,** *548 (1950)*) have compared the nutritive values of glucose, lactose, and corn syrup as carbohydrate components in synthetic milk for young calves. Lactose was selected for study because it is the natural source of carbohydrate for newborn mammals, and corn syrup was included in the study because it is frequently used as a carbohydrate addition in milk formulas for infants.

One female calf and 17 male calves were obtained from the college experimental herd. They were removed from their dams twelve hours after parturition and after fourteen to twenty-four hours of starving were started on the synthetic milk diets. The calves were fed twice daily via nipple pail at a rate calculated to meet the allowance recommended by the National Research Council.

The control ration consisted of the following: glucose 60; casein 25; lard 10; and salts 5 per cent. In addition, each calf received 70,000 I.U. of vitamin A and 10,000 I.U. of vitamin D weekly and the following supplement once daily: thiamine 20; riboflavin 20; calcium pantothenate 20; niacin 20; para-aminobenzoic acid 20; pyridoxine 5; vitamin K 10; biotin 1; inositol 200; and choline chloride 3000 mg.

Three corn syrup-containing rations were tried. They differed from the control ration in that 10, 30, or 45 per cent of the ration was composed of corn syrup in place of equal amounts of glucose. Three lactose-containing rations were studied. They were similar to the control ration except that 5, 10, or 30 per cent of the glucose was replaced by equal amounts of lactose. The synthetic milk was fed as a liquid containing approximately 11 per cent solids.

All calves drank the synthetic milk readily throughout the thirty-one-day experimental period. Within two to four days after being placed on experiment, calves on the control ration and those on rations containing corn syrup developed soft, and in many cases semiliquid, feces. Those on corn syrup rations were most seriously afflicted. Calves on lactose rations maintained normal feces throughout the experiment. Bloat occurred in 2 calves receiving the control ration, and one calf receiving the ration containing 10 per cent lactose died of acute bloat.

The 6 control calves gained an average of 9.33 pounds in body weight in thirty-one days, which was an 8.13 per cent increase over the starting weight. The 6 calves receiving the corn syrup rations gained an average of 8.66 pounds, or a 10.23 per cent increase over the starting weight. Those on the ration containing 45 per cent of corn

syrup actually lost weight. The 6 calves receiving lactose rations gained an average of 18.66 pounds, or a 25.92 per cent increase over the starting weight.

The efficiency of feed utilization (average gain in pounds per pound of food consumed) was 0.234, 0.142, and 0.384, respectively, for the controls, those receiving corn syrup rations, and those receiving lactose rations. Blood values for hemoglobin, hematocrit, plasma calcium, inorganic phosphorus, magnesium, and ascorbic acid were within the normal range in all the calves. The rumen contents were examined for microorganisms. The rumen populations varied a great deal within and between groups so that no significance could be attached to these data.

Ten of the 18 calves were subjected to postmortem examination. Congestion and consolidation of lung tissue were the most common findings and apparently were least severe in the calves receiving lactose rations. Ulceration of the pylorus, petechial hemorrhages of the abomasum, and patchy congestion of the intestinal tract occurred in the animals receiving corn syrup rations and those receiving the control ration. These lesions were likewise observed in the calves receiving lactose rations but were less severe.

Focal interstitial nephritis occurred irrespective of diet. Although growth responses by the calves receiving lactose rations were considered satisfactory, these calves were not considered to be normal because of the respiratory disturbances as well as other visible abnormalities noted at autopsy. However, it was emphasized that the physical condition of the calves receiving rations containing lactose was superior to that in the other groups. Thus lactose appeared to possess favorable activity in the nutrition of young calves which could not be equaled by either glucose or corn syrup.

In a second paper these authors extended their investigations to determine the influence of lactose on the nutritive value of starch and corn syrup (R. J. Flipse, C. F. Huffman, C. W. Duncan, and H. D. Webster, *J. Dairy Sci.* **33,** *557 (1950)*). In this study the control ration was similar to that used in the previous study except that 45 per cent of starch was included at the expense of an equal amount of glucose. The carbohydrate components of the two experimental rations were as follows: ration SL:45 per cent starch, 10 per cent lactose, and 5 per cent glucose; ration KL:45 per cent corn syrup, 10 per cent lactose, and 5 per cent glucose.

Nine calves were used, 3 on each ration. Soft feces were excreted by the control group within two to four days after the beginning of the experiment. Although diarrhea was noted, it was much less severe in the group receiving starch with lactose (ration SL). Diarrhea occurred only infrequently in the group receiving the corn syrup with lactose (ration KL). This was in marked contrast to the severe diarrhea noted with corn syrup alone in the previous experiment. Average gains in body weight for the thirty-one-day feeding period were 28.33, 24.67, and 14.00 pounds for the KL, SL, and control rations, respectively. The best weight gains were also the most efficiently produced gains.

In search for a possible explanation for the varying responses to the carbohydrate used in these studies, a test meal containing a single carbohydrate source (glucose, lactose, corn syrup, or starch) was fed and blood sugar concentration determined over the subsequent eight-hour period. These test meals were fed to calves 28 to 35 days old. With starch there was no increase in blood sugar. The other three carbohydrates produced rapid increases in blood sugar with no differences evident during the first thirty minutes after feeding. Thereafter the differences were striking.

The corn syrup test meal produced maximum blood sugar concentration one hour after feeding (approximately 80 mg. per 100 ml. of blood). The peaks after glucose and lactose feeding were reached four hours

after feeding and were approximately 140 mg. per 100 ml. of blood. The glucose meal, however, resulted in a much more rapid decline in blood sugar after the peak was reached than did the lactose meal. Eight hours after feeding glucose the blood sugar concentration had nearly returned to normal (65 mg. per 100 ml. of blood), while eight hours after lactose feeding the blood sugar concentration was still about 115 mg. per 100 ml. of blood.

The poor physical condition and the diarrhea which resulted when calves were fed a synthetic milk containing glucose as the only carbohydrate component, in the present study, are in disagreement with the results reported by Wiese and associates (*loc. cit.*). These workers reported that a synthetic milk made up of casein, lard, salt, and glucose produced normal growth and healthy calves.

The studies by the Michigan workers emphasize that lactose is superior to glucose, corn syrup, or starch as a carbohydrate in the ration of young calves and that as little as 10 per cent of lactose in the ration greatly improves rations containing starch or corn syrup. These results also provide a basis for the common practice of including lactose, in the form of dry whey or nonfat milk solids, in calf starter rations.

RIBOFLAVIN INTAKE VERSUS EXCRETION

Diagnosis of ariboflavinosis (riboflavin deficiency) in man is usually based on one or more of the following: evidence of a deficient food intake, visible deficiency lesions, or the level of urinary excretion of the vitamin. Physical manifestations, such as angular stomatitis or corneal vascularization are nonspecific for riboflavin deficiency and may appear in other deficiencies. Accurate estimates of previous riboflavin intake are usually not easily obtainable in patients.

Workers at the Elgin State Hospital, Elgin, Illinois, have conducted extensive investigations to determine whether urinary excretion of riboflavin could be correlated with symptoms of ariboflavinosis or with the level of dietary intake of the vitamin. Data were obtained on twenty-four-hour urine samples and on samples obtained during the four-hour period following administration of 1 mg. of riboflavin. The results obtained by both technics paralleled each other, so for the purpose of this review the data given will be limited to the twenty-four-hour samples.

The first project in the six-year study dealt with riboflavin and thiamine requirements in man and was reported by M. K. Horwitt, E. Liebert, O. Kreisler, and P. Wittman (*National Research Council Bull.* **116** (*1948*); *Nutrition Reviews* **7,** *264* (*1949*)). The second project concerned correlation of riboflavin intake with symptoms of deficiency and level of urinary excretion (Horwitt, C. C. Harvey, O. W. Hills, and Liebert, *J. Nutrition* **41,** *247* (*1950*)). Subjects who were receiving eight different levels of riboflavin were included in this study, their daily intakes ranging from 0.55 to 3.55 mg. A detailed description of the dietary management of the subjects was also reported (Horwitt, G. Sampson, Hills, and D. L. Steinberg, *J. Am. Dietet. Assn.* **25,** *591* (*1949*)).

A diet adequate in other essential factors and providing 1.10 mg. of riboflavin daily was fed for a three-month preliminary period to 30 male subjects. Fifteen of these subjects were then placed on a depletion diet providing 0.55 mg. of riboflavin. The depletion diet was fed for periods ranging from nine to seventeen months. Fourteen of the original subjects were fed the same depletion diet plus 2.00 mg. of riboflavin (daily riboflavin intake, 2.55 mg.). This supplement was increased to 3.00 mg. (daily

riboflavin intake, 3.55 mg.) after fifteen months.

To obtain information on recovery following riboflavin depletion, 14 of the subjects who had been on the depletion diet for nine to seventeen months received supplements of riboflavin as follows: 4 received 6 mg. once daily; 3 received 2 mg. three times daily; 3 received 2 mg. twice daily; and 4 received 2 mg. once daily.

Initially the subjects excreted an average of 400 micrograms of riboflavin daily via the urine. At the end of the three-month preliminary period (1.10 mg. daily riboflavin intake) the 15 subjects selected for the depletion study were excreting an average of 112 ± 75 micrograms of riboflavin daily. This dropped to 49 ± 19 micrograms daily within seven days after the subjects were placed on the depletion diet (0.55 mg. daily riboflavin intake). They remained at this level of urinary excretion of the vitamin for several months. After nine months on the depletion diet the urinary excretion of riboflavin had decreased to 37 ± 6 micrograms daily, and no significant change occurred during the remaining eight months.

Riboflavin deficiency symptoms (angular stomatitis) appeared within four months in one of the subjects on the depletion diet. This subject was excreting between 35 and 40 micrograms of riboflavin daily via the urine when symptoms developed. In no cases were clinical symptoms of ariboflavinosis observed in subjects excreting more than 40 micrograms of riboflavin daily. A detailed discussion of the clinical examinations was not given, but the authors point out that a diet low in riboflavin, with consequent low riboflavin excretion, does not guarantee uniform development of visible symptoms of riboflavin deficiency.

There was no indication that the tissues of depleted subjects could absorb riboflavin more efficiently when smaller or divided doses were given as contrasted with a single large dose. Assuming urinary excretion to be an index of tissue saturation, these workers found that a single dose of 6 mg. of riboflavin daily in depleted subjects was as effective in increasing urinary excretion as were three 2 mg. doses given at four-hour intervals. A period of approximately fifteen days was required to saturate the tissues of riboflavin-depleted subjects when a daily supplement of 6 mg. was administered.

Data on urinary riboflavin excretion were also given on 53 patients receiving hospital diets that supplied daily riboflavin intakes of 0.75, 0.85, 1.60, and 2.15 mg. These levels of intake together with the levels included in the depletion study (0.55, 1.10, 2.55, and 3.55) permitted comparisons of urinary excretion of riboflavin at eight different levels of intake. Except for the subjects on the depletion diet the data given represented levels of riboflavin excretion reached in ten weeks or less on the designated riboflavin intakes.

Subjects receiving 1.10 mg. of riboflavin or less daily were excreting less than 10 per cent of the intake. Subjects receiving 1.60 mg. or more daily were excreting from 26 to 48 per cent of the intake. The daily urinary excretions were 97 ± 62 and 434 ± 185 micrograms, respectively. Thus there was a marked increase in the percentage of intake that was excreted when the intake was raised from 1.10 to 1.60 mg. daily. From this the authors concluded that the critical riboflavin requirement was somewhere between 1.10 and 1.60 mg. daily for an adult male subsisting on about 2200 calories daily. This result, reported in detail by M. K. Horwitt and co-workers (*J. Nutrition* **39,** *357 (1949)*) is in accord with reports previously reviewed which suggested a minimum riboflavin requirement for adults of approximately 0.50 mg. daily per 1000 calories (*Nutrition Reviews* **5,** *60 (1947)*).

The Elgin Hospital workers observed that individual variations in urinary excretion of riboflavin were large during early riboflavin deficiency, and it was therefore difficult to designate definite excretion levels as associated with ariboflavinosis. However, under

the conditions of their studies, in which the subjects were well protected against adverse environmental factors, any twenty-four-hour excretion of less than 100 micrograms of riboflavin was considered as indicative of less than desirable dietary intakes. Levels of excretion below 50 micrograms daily were considered as evidence of prolonged limited dietary intake, with clinical symptoms of ariboflavinosis developing in some cases.

The results of another group of workers are not in strict agreement with those of the Elgin Hospital workers. H. Oldham, B. B. Sheft, and T. Porter (*J. Nutrition* **41,** *231* (*1950*)) studied riboflavin intake and excretion during pregnancy and noted that the twenty-four-hour urinary excretion of riboflavin during the latter months of pregnancy in 2 subjects was 120 and 160 micrograms on respective daily intakes of 0.49 and 0.47 mg. These excretion levels are more than twice as large as those reported by the Elgin Hospital workers in subjects on a daily riboflavin intake of 0.55 mg. Oldham and associates were comparing the urinary excretion of riboflavin and thiamine in pregnant and nonpregnant women and came to the conclusion that the requirements for these vitamins were not increased during pregnancy. Evidence for this conclusion was based on the observation that the point of minimum excretion of riboflavin, for example, was obtained in pregnant women on intakes no higher than those of nonpregnant women.

The problem of recognition of specific ariboflavinosis in man and the interpretations of the relation between dietary intake and urinary excretion have received considerable attention in recent years (*Nutrition Reviews* **3,** *235* (*1945*); **4,** *310* (*1946*); **5,** *60*, *227* (*1947*); **7,** *38* (*1949*); **8,** *133* (*1950*)). The Elgin Hospital reports are noteworthy because diet and urinary excretion records were obtained on many of the subjects for consecutive periods of four to six years. More well-designed and extended studies of this type are needed if relationships between intake and urinary elimination of essential dietary factors are to be clarified.

MALNUTRITIONAL BLINDNESS

Blindness that is the result of a specific infection has for the most part been controlled throughout the world. Of the preventable types of blindness, perhaps malnutritional blindness is the most important. C. J. Blumenthal (*South African Med. J.* **24,** *191* (*1950*)) summarized a ten-year clinical study of malnutritional keratoconjunctivitis as seen in the South African Bantu. About ten years ago, after starting practice in Johannesburg, the author first saw an instance of spontaneous prolapse of the iris in a 6-month-old Bantu infant. The parents of the infant stated that there had been no injury to the eye, but to the author the corneal perforation wound, with its clean, mushroom-like prolapse of the iris, appeared exactly like that caused by trauma. Later, as similar instances were encountered, it became obvious that he was seeing a disease which to his knowledge had not been described before.

Apparently, malnutritional keratitis is a disease of the poor, and it seems to be totally the result of poor nutrition in a rural population, backward in their knowledge of soil husbandry. The author believes that malnutritional keratitis is by far the commonest cause of preventable blindness in South Africa, and that it ranks with any of the other important causes of blindness, such as glaucoma, cataracts, trauma, etc. In contrast to these other diseases, malnutritional keratitis is chiefly a disease of the young. The author states that most of these African children, with the usual mental

apathy which accompanies a low nutritional level, are not fit subjects for the labor market and, since they live a useless life, they disappear to the kraal, where they succumb to an early and needless death.

From the author's observations, he believes that malnutritional keratitis is related to nutrition purely in the qualitative and not in the quantitative sense. He has never seen it occur in an extremely emaciated Bantu child, and states very bluntly that "it does not occur as a result of starvation." Most of the children in whom it is seen are pudgy and fat, and have been on nearly a purely carbohydrate diet without protein or vitamins.

In the early and treatable forms of this conjunctivitis, a dramatic change follows administration of mixed vitamins. From a rather listless and apathetic, miserable creature the child is transformed in a few days into a cheerful, energetic individual. The running nose and watery eyes dry up and the photophobia disappears. A rather hopeless looking cornea, which may even have perforated, responds in these young persons in an extraordinary manner. Apparently, local treatment to the eye is unnecessary, unless perforation or prolapse is threatening.

The author discusses several clinical types of corneal softening or liquefaction, which are technical and of interest only to the ophthalmologist. These conditions are outlined in some detail in the article. There is a spontaneous prolapse in which the cornea dissolves insidiously at one small point, without a sign in the eye to attract superficial attention. The usual point for corneal dissolution is in the lower segment. The prolapsed knuckle of the iris usually is perfectly round, much like a button mushroom, and about 1 to 2 mm. in diameter. Several rather dramatic photographs are shown in the article. The eye is not inflamed. This form has been seen only in infants and young children. The author has seen hundreds of patients of this type without ever seeing a sympathetic ophthalmitis.

Another variation is the nipple or teat type, in which the protrusion often is enormous. Still another type is corneal softening with secondary infection. Catarrhal keratoconjunctivitis is another type, characterized by excessive lacrimation, increased production of mucus, and severe photophobia. This is the type that reacts dramatically to good food and vitamins. It apparently is one of the early types, and one cannot tell into which of the above-mentioned types it will develop.

The author gives an interesting account of an ophthalmologic condition which apparently in some stages can be corrected by administration of a well-balanced diet and vitamin supplements. However, no scientific data are given in regard to doses required or the types of food indicated, and no scientific control study has been attempted. The clinical observations, however, are important. It must remain for those interested in nutrition to carry out the scientific studies in the future.

DEFICIENCIES OF ASCORBIC ACID AND PTEROYLGLUTAMIC ACID IN ANEMIA

Macrocytic anemia associated with megaloblastic arrest of the bone marrow in infants has been described in recent years (*Nutrition Reviews* **4**, *338* (*1946*)). The peripheral blood picture is not always characteristic and diagnosis is, therefore, dependent on demonstration of typical changes in the bone marrow. The disease occurs most commonly in infants from 5 to 11 months of age and has been termed megaloblastic anemia in infancy. The bone marrow defect and changes in the peripheral blood are corrected by treatment with pteroylglutamic acid or liver extract.

Occurrence of the disease would seem to indicate interruption of normal hematopoie-

sis, either because of inadequate intake of factors essential to blood formation or metabolic defects interfering with utilization of these materials. Milk, which is the sole or chief article of the diet in early infancy, is found to contain both pteroylglutamic acid and vitamin B_{12}, the two active hematopoietic factors which have thus far been isolated from liver. Present evidence would make it appear unlikely that a simple or uncomplicated deficiency of these nutrients accounts for the occurrence of megaloblastic anemia in infancy.

Clinical reports record frequent history of dietary inadequacy of ascorbic acid in many infants with the disease (R. A. Aldrich and E. N. Nelson, *J.-Lancet* **67,** *399 (1947)*). In addition, manifestations of scurvy have been observed in certain patients (W. W. Zuelzer and F. N. Ogden, *Am. J. Dis. Child.* **71,** *211 (1946)*). In other areas of investigation it has been noted that scurvy may be accompanied by an anemia of the macrocytic type (*Nutrition Reviews* **4,** *328 (1946)*), and that ascorbic acid and pteroylglutamic acid are importantly related in their roles in intermediary metabolism (*Ibid.* **8,** *260 (1950)*).

The role of ascorbic acid deficiency in the pathogenesis of megaloblastic anemia has been studied experimentally by C. D. May, E. N. Nelson, C. U. Lowe, and R. J. Salmon (*Am. J. Dis. Child.* **80,** *191 (1950)*). The protocol of the experiment included feeding various milk formulas to *Macacus rhesus* and *Macacus cynomolgus* monkeys, of an age from newborn to near maturity. These species require a dietary source of ascorbic acid. The milk component of the diets was supplied from one of a variety of sources, including human milk, cow's milk (fresh pasteurized, evaporated, or powdered), evaporated goat's milk, and two commercial infant food preparations. Milk formulas were boiled or treated with copper to destroy all but small traces of ascorbic acid. By analysis, feedings contained from 0.2 to 0.7 mg. of ascorbic acid per 100 ml. The concentration of pteroylglutamic acids in the formulas varied from 0.2 to 0.4 microgram per 100 ml., representing a level of dietary intake which is admittedly low. Protein contents of the diets were in the range from 1.4 to 3.5 g. per 100 ml. All formulas were supplemented with vitamin A, vitamin D, thiamine, riboflavin, niacin, and ferrous gluconate in quantities adequate to meet daily needs. Monkeys were examined at intervals for evidences of scurvy. Frequent blood hemoglobin determinations and bone marrow aspirations were done.

In control periods monkeys were fed one or another of the infant-feeding formulas ad libitum and 50 mg. of ascorbic acid were added to the daily diet. Megaloblastic anemia did not occur in any of 10 control animals observed for periods from two and one-half to eighteen months.

Typical manifestations of scurvy developed in all animals fed milk formulas without addition of ascorbic acid. This occurred regardless of the milk source or the protein content of the diet. The period between beginning of the experimental diet and appearance of first signs of scurvy varied from fifty to three hundred and two days.

Megaloblastic anemia occurred in 16 animals not receiving supplements of ascorbic acid. Evidences of the characteristic change in bone marrow were usually obtained several days after the appearance of first signs of scurvy. Marked and rapid decrease in blood hemoglobin concentration was associated with megaloblastic arrest in bone marrow. Only one monkey fed the deficient diet failed to develop megaloblastic anemia. This animal received 5 mg. of pteroylglutamic acid orally once a week.

Loss of appetite occurred as an early manifestation of scurvy and food intakes of all of the deficient animals were markedly decreased. In an effort to determine whether starvation had a role in causing the hematologic defect, one monkey was given one of the milk diets supplemented with ascorbic acid, but allowing only one-tenth of the

caloric intake consumed when the animal was fed ad libitum. After six weeks this animal had lost 25 per cent of its body weight but neither anemia nor megaloblastic changes in the bone marrow developed. It should be pointed out, however, that according to the authors' data megaloblastic anemia did not occur in any animal in a period shorter than sixty days on a diet deficient in ascorbic acid.

After it had been demonstrated that megaloblastic anemia could be produced experimentally in the monkey it was then possible to determine the effects of various vitamins on the hematologic defect. During the testing of therapeutic effects the animals continued to consume diets which had resulted in production of anemia. Provision of ascorbic acid to deficient animals permitted gradual return of the marrow to normal. The anemia and marrow defect were more quickly corrected by oral or intramuscular administration of pteroylglutamic acid. This effect was observed whether or not ascorbic acid was also provided. Vitamin B_{12} given intramuscularly did not alter the megaloblastic character of the marrow but when administered in combination with ascorbic acid, the marrow quickly reverted to normal.

An attempt was made to determine whether pteroylglutamic acid or vitamin B_{12} might prevent occurrence of megaloblastic anemia when administered to monkeys on ascorbic acid-deficient diets. Five micrograms of pteroylglutamic acid given by mouth daily did not protect against development of the characteristic anemia in 2 monkeys, but a larger quantity, 5 mg. per week, did prevent it in one animal. Vitamin B_{12} had no effect in obviating the occurrence of this anemia.

Since it was possible to prevent development of megaloblastic anemia in monkeys fed an ascorbic acid-deficient diet by providing supplements of pteroylglutamic acid, it seemed likely that the immediate cause of the marrow defect was deficiency of pteroylglutamic acid. In support of this was the finding that the concentration of pteroylglutamic acid in livers of anemic animals was much reduced as compared with control animals. However, excretion of the vitamin in the urine and feces was similar in monkeys in control and experimental groups. These observations are of interest since they indicate that megaloblastic anemia occurs in monkeys on diets deficient in pteroylglutamic acid only when the diet is also deficient in ascorbic acid.

Other efforts to produce experimental deficiency of pteroylglutamic acid in experimental animals have usually succeeded only when an antagonist of the vitamin or a sulfonamide was added to the diet. In this experimentally produced hematologic defect may reside opportunities for investigating more fully the metabolic interrelationships between pteroylglutamic acid, vitamin B_{12}, and ascorbic acid.

There is indirect evidence which might give support to the concept that these observations pertaining to experimental anemia produced in monkeys apply in megaloblastic anemia in infants. As stated earlier, it has been reported that certain infants with megaloblastic anemia have had inadequate dietary intake of ascorbic acid. Moreover, the age at which megaloblastic anemia and scurvy occur most commonly is similar, with peak incidence in infants of 5 to 11 months. Again by way of indirect evidence is cited the nearly complete disappearance of megaloblastic anemia in infants in the United States within the past two years. This has occurred coincident with introduction of a procedure adding ascorbic acid to two commonly used proprietary infant foods. Earlier, many infants who developed megaloblastic anemia had been on formulas containing these foods.

Anemia usually accompanies the clinical syndrome of scurvy, but is variable in type. It is wholly possible that those individuals with scurvy and with manifestations of macrocytic anemia may have combined deficiencies of ascorbic acid and pteroylglutamic acid.

MODES OF ACTION OF PEPSIN ON PROTEINS

The study of pepsin, including its mechanism of action, has been one of the classic problems of biochemistry and nutrition. Discovered by Schwann in 1836, pepsin was isolated by Langley in 1882 and crystallized by Northrop in 1930. Its occurrence in nature and the kinetics of its action at various pH conditions and temperatures have been the object of numerous publications and are relatively well known. While the specificity and mode of action of pepsin have also been studied extensively, particularly in the past fifteen years, a recent paper by P. Desnuelle, M. Rovery, and G. Bonjour (*Biochim. et biophys. acta* **5,** *116 (1950)*) indicates that much remains to be explained and that great caution is needed when theorizing on the subject.

For a long time it had been considered sufficient to classify pepsin as a "proteinase" (thus named in contradistinction to peptidases, which attack simple peptides) characterized by the fact that it attacked nearly all native proteins and transformed them into "proteoses" and "peptones." Starting in 1937 M. Bergmann and his collaborators published a series of papers (*see Advances in Enzymol.* **2,** *49 (1942)*) in which it was shown that pepsin could attack simple peptides provided they had the proper configuration. Their concept was that there was a specificity of substrate allowing pepsin action independently of the size of the substrate molecule. Pepsin (along with trypsin and chymotrypsin) was classified as an "endopeptidase" and further characterized by the following substrate specificity (also typical of spleen and kidney pepsinases or cathepsin I): presence of at least two carboxyl groups in the peptide, absence of a proximal free-amino group, and the presence of an aromatic residue (phenylalanyl or tyrosyl) on the amino side of the linkage to be attacked. However, C. R. Harington and R. V. Pitt Rivers (*Nature* **154,** *301 (1944)*) later showed that tyrosylcysteine, although not fulfilling all of Bergmann's criteria, was in fact split by pepsin.

Desnuelle and co-workers (*loc. cit. 1950*) have reopened the problem of the specificity of peptic hydrolysis by studying the action of pepsin not on simple peptides but on complex proteins, namely, horse globin and egg albumin. They attempted, in so far as is now possible to follow the course of the hydrolysis. Thus the alpha-amino groups liberated during the reaction were characterized by Van Slyke's method, periodic oxidation, and Sanger's fluorodinitrobenzene technic. The latter two methods, relatively recent, may require a description: Periodic oxidation allows the characterization of hydroxy amino acids (serine and threonine). While its import is thus limited to these two amino acids, it permits, by using the ratio of the "liberation" of these two amino acids to total amino acid produced, characterized by the classic technic of Van Slyke, discernment of patterns in the course of the hydrolysis. For example, it was shown that in the case of globin, hydrolysis of the "serine linkages" (linkages adjacent to a seryl residue) proceeds at approximately twice the normal rate. In the case of albumin, hydrolysis of serine linkages proceeds at the average rate, hydrolysis of threonine linkages is extremely slow. The fluorodinitrobenzene (FDNB) technic on the other hand, while somewhat laborious is of a much more general application. At proper intervals the enzymatic hydrolysates are treated with FDNB and the proper reagents. Free-amino groups combine with FDNB and after each hydrolysis of the residue can be characterized individually by chromatographic analysis (F. Sanger, *Biochem. J.* **39,** *507 (1945)*).

Generally speaking, it was found that peptic hydrolysis proceeded differently, in the case of globin, as contrasted with albumin. In the case of globin, peptic hydrolysis proceeded in two successive phases. During the first phase very large peptides and duodecapeptides (on the average) are formed

simultaneously from the protein chains. The large peptides are themselves rapidly transformed into duodecapeptides, which can therefore be considered as typical of this particular stage. This first phase of degradation is rapid. Characterization of terminal amino acids shows that this degradation occurs according to certain rules of specificity: pepsin during this phase breaks up preferentially those bonds in which alanine, phenylalanine, leucine, and serine are involved through their amino groups. Unfortunately, the lack of methods allowing the characterization of the carboxyl terminal groups prevented collection of information on other (carboxyl) specificities. During a second phase of the hydrolysis the peptides undergo a further degradation down to the tetrapeptide (average) stage. This phase proceeds very slowly and does not seem to present any specificity (at least so far as the amino side of linkages is concerned).

On the other hand, in the case of albumin there does not seem to be any general formation of large peptides. A first phase proceeds rapidly to the hexa- or pentapeptide stage. No specificity can be demonstrated in the hydrolysis of individual linkages. After this rapid initial degradation very little further hydrolysis occurs although eventually again an average of four amino acids per peptide residue (representing a decrease of one to two) is reached. No specificity can be demonstrated in this second phase either.

Thus it seems clearly demonstrated that the nature of the protein substrate is a very important factor in determining the mode of action of proteolytic enzymes. The lag and order of liberation of peptides, and therefore eventually of amino acids, may differ considerably between albumins and globulins and possibly between diverse albumins or diverse globulins. Now that the timing of absorption of amino acids has been shown to be essential for their proper utilization, the differential treatment of diversified proteins by proteolytic enzymes will no doubt be shown to be important in the determination of the biologic value of proteins and protein mixtures.

ASSESSMENT OF THE AMINO ACID COMPOSITION AND NUTRITIONAL VALUE OF PROTEIN

In the past quarter of a century many reports have appeared in the literature on the biologic value, nutritional value, and dietary usefulness of proteins. During the past few years numerous publications have dealt with the complete or almost complete amino acid composition of various proteins. It must be recognized that both types of studies have shown not only great differences in methods and technics but also important inconsistencies and divergence of results. The Bureau of Biological Research of Rutgers University decided, therefore, that a comprehensive testing program where methods and results of a large number of laboratories could be compared would be a worth-while undertaking. This program was executed in the period 1946 to 1950 and a report of it has been published by J. B. Allison, R. H. Barnes, R. A. Harte, and W. H. Cole ("Report on Cooperative Determinations of the Amino Acid Content and of the Nutritive Value of Six Selected Protein Food Sources," *Bureau of Biol. Res., Rutgers University, New Brunswick, N. J. (1950)*) which covers both amino acid analyses and determination of biologic values.

The proteins and protein-containing foodstuffs were selected on the basis of wide range of probable biologic value, easy availability, low cost, and keeping quality. Whole defatted dried egg, dried egg albumin, defatted dried beef muscle, "Labco" casein, partially defatted peanut flour, and wheat germ gluten meal were studied. Twelve well-known academic and industrial laboratories

agreed to determine the amino acid content of the six proteins, of the reference hydrolysates of these proteins and, as reference standards, of a mixture of purified amino acids. The methods used were the methods regularly employed by them.

Two laboratories used chemical methods; nine laboratories employed microbiologic methods, making use of thirteen microorganisms. Sixteen amino acids were determined in the protein analyses: arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine, and valine. In addition cysteine and serine were determined among the pure amino acids of the reference mixture.

Considerable variation in over-all accuracy was encountered even in the analysis of the reference mixture. In some cases (e.g., glutamic acid, isoleucine, methionine, and valine) consistently high values were encountered. Tryptophan and cysteine determinations showed consistently high algebraic and absolute deviations (15 and 31.2 per cent, respectively). Other absolute mean deviations varied between 2.4 and 8.2 per cent. So far as the analyses of the proteins were concerned variations were even more considerable, particularly for some amino acids. For tryptophan, the extreme case, some determinations varied from 1 to 3 for the same protein. Generally speaking, it was found that the errors intrinsic in the actual analytical system of determinations were so much larger than those inherent in the differences in hydrolysis technics as to make the latter negligible. No opinion could be formed from this study on the relative merits of chemical and microbiologic studies.

Twenty laboratories, seven academic and thirteen industrial, undertook to determine the nutritive value of the six proteins by methods regularly used by them. These methods were: growth of young animals; nitrogen balance, repletion and double depletion in adult rats; reproduction and lactation.

The determinations of biologic values based on growth studies utilized rats, dogs, and mice. Digestibilities, weight gains, and efficiencies of protein conversion were measured. The average order of sequence in the rat was found to be: egg white, whole egg, beef muscle, casein, peanut flour, and wheat gluten. However, considerable differences were encountered between laboratories in the order of sequence of the four best proteins. More consistent differences could be demonstrated when efficiency of conversion of protein to body weight in the rat was used as the criterion. The order of values was again egg white, whole egg, beef muscle, casein, peanut flour, and wheat gluten. In the mouse-growth test egg white was second to whole egg. Otherwise the order was the same. Finally, in the dog-growth method egg white was poorest of all instead of best of all as in the rat.

When adult animals were used, digestibilities were first determined. Values for various proteins were found to vary between 95.2 and 99.5 per cent. For the determinations of biologic values based on nitrogen balance studies, experimental subjects were men, dogs, and rats. Whole egg was found to have the greatest value of all protein sources in man, while egg white headed the list in the dog and rat (perhaps due to its proportionately higher content of methionine). A relatively high value was found for wheat gluten when the rat was used as experimental subject, but neither men nor dogs were used. Because a low value had been found for this protein for the rat if growth or repletion was used for the determination, this result may support the finding that lysine, essential for tissue growth in this animal, is not an essential amino acid in the adult rat (H. H. Mitchell, *Arch. Biochem.* **12,** *293 (1947)*).

The other methods used led to similarly diversified results. Repletion in the rat and dog according to the criteria of average total

weight gain, protein efficiency, carcass protein gain, total circulating plasma protein gain, total circulating hemoglobin, and liver protein gain, led to the following sequence for the rat: egg white, whole egg, beef muscle and casein, peanut flour, and wheat gluten. In the dog again, egg white had a very poor value. The double depletion method in the dog suggested that diets could be developed which promote the production of one type of body protein more than another. Casein was particularly excellent in promoting both plasma proteins and hemoglobin formation. Data on reproduction did not permit adequate comparison; they only indicated the low value of wheat gluten. Data on lactation put beef muscle and whole egg first, then egg white and casein, peanut flour, and gluten.

This important and painstaking study thus yields many significant results. It again illustrates the difficulties entailed in routine determination of amino acids and emphasizes the necessity for careful comparison of values obtained by different laboratories. At a time when establishment of food composition tables giving the amino acid content of foodstuffs is seriously considered, such a demonstration of the difficulties of this task is useful.

So far as determinations of biologic values are concerned, this study emphasizes not only the difficulties in choosing adequate methods, criteria, and experimental animals, but also the fact that the biologic value of a protein is not a fixed quantity but varies according to the kind of animal chosen, to its physiologic state, and to the particular nutritional tasks which are chosen as criteria.

PHOSPHORUS METABOLISM IN BRAIN

On the basis of the correlation of chemical analyses of brain tissue with accompanying functional changes, it seems reasonable to believe that the metabolic activity of this organ varies with physiologic activity. With reference to changes in phosphorus metabolism, R. M. C. Dawson and D. Richter (*Am. J. Physiol.* **160,** *203* (*1950*)) have shown that a rapid breakdown of phosphocreatine and an increase in the hexose phosphate fraction of brain occur in convulsions, changes possibly associated with mobilization of carbohydrate calories. Though the rate of entry of radioactive phosphorus into the brain is relatively slow (G. Hevesy, *J. Chem. Soc., p. 1213* (*1939*)) and though phosphorus retention by the brain is the poorest of any organ studied (W. E. Cohn and D. M. Greenberg, *J. Biol. Chem.* **123,** *185* (*1938*)), synthesis of phosphate esters, studied with radioactive phosphorus, appears to be relatively rapid (O. Lindberg and L. Ernster, *Biochem. J.* **46,** *43* (*1950*)).

In a later report (R. M. C. Dawson and D. Richter, *Proc. Roy. Soc. B* **137,** *252* (*1950*)) phosphorus metabolism was investigated by following the uptake of the radioactive isotope P^{32} from the blood into the several phosphorus-containing fractions of the brain.

Radioactive phosphate was given to 3- to 6-month-old mice either by subcutaneous or intraperitoneal injection, in amounts of 0.2 ml. containing 0.02 mg. of phosphorus as dihydrogen sodium phosphate and 10 millicuries of radioactive phosphorus. After allowing three hours for phosphorus exchange, the mouse was killed, a sample of blood secured, and the entire brain removed for study.

By suitable procedures described in detail, the phosphorus compounds of the brain were separated into three fractions: (I) acid-soluble fraction containing nucleotides, inorganic phosphates, and phosphate esters soluble in 5 per cent aqueous trichloroacetic acid; (II) nucleoprotein fraction containing ribo- and desoxyribonucleic acids and a small amount of phosphoprotein; (III) phospholipid fraction containing lecithins,

cephalins, and sphingomyelins. The specific activities of these fractions were determined by measuring the ratio of the radioactive phosphorus to the total phosphorus. To eliminate individual variation among animals, the rate of turnover was estimated by using a specific activity ratio in which the specific activity of one fraction was always equated with that of a reference fraction, thus obtaining a ratio which is independent of the absolute amount of radioactive phosphorus and which indicates the rate of transfer of the isotope into the fraction. Suitable corrections for radioactive decay, for the solvent absorption of beta particles, and for the blood content of the brain were made.

Time-serial studies were made following injection of the radioactive phosphate in order to determine the rate of passage of the isotope into the various brain fractions. The rate of passage from the acid-soluble fraction of blood to that in the brain was not constant but diminished after the first hour. On the other hand, the activity ratios indicate that the nucleoprotein fraction and the phospholipid fraction show a relatively high metabolic activity in so far as their uptake of radioactive phosphorus is concerned. These fractions have generally been regarded as relatively inert chemically.

Having secured activity ratios at the three-hour interval for a series of normal mice, the relation of various physiologic states to phosphorus metabolism of brain was examined. Light nembutal anesthesia produced activity ratio values indicating that there was reduced phosphorus turnover in the nucleoprotein and phospholipid fraction. If body temperature was allowed to drop during the anesthesia, all three fractions of the brain showed reduced phosphorus metabolism. When insulin was administered immediately before the radioactive phosphorus, the turnover of phosphorus in the phospholipid fraction was greatly reduced. Electric stimulation severe enough to produce convulsions produced a significant drop in phosphorus metabolism in the phospholipid fraction but no alteration in the other two fractions.

To study emotional excitement mice were slowly rotated in a drum. If the mice had been trained to this procedure for three weeks prior to administration of the radioactive phosphorus, no effects on phosphorus metabolism of the brain were noted; if the mice had not been trained, a marked drop in phosphorus turnover in the phospholipid fraction resulted.

The foregoing observations may serve to stimulate speculation regarding the functions and chemical inertness of the various phosphorus compounds in brain. It would appear that the traditional view that phospholipids are structural elements and hence more or less nonreactive chemically, needs revision in the light of such studies.

RUMEN MICROORGANISMS IN YOUNG CALVES

Investigations have been under way for several years at the Ohio Agricultural Experiment Station to determine the significance of rumen microorganisms in the nutrition of young calves. These studies were prompted by inadequate explanations for the causes of digestive disturbances and other diseases in calves during the first several weeks of postnatal life.

The Ohio workers, W. D. Pounden and J. W. Hibbs (*J. Dairy Sci.* **30,** *582 (1947)*) observed that rumen fauna and flora similar to those present in mature cattle were not established in calves until they were several weeks old. Direct inoculation of organisms from cows into the rumens of calves was a successful means of establishing these organisms in some calves. Later it was found that the success or failure of the inoculation procedure was largely dependent upon the

type of ration fed to the calves (*Ibid.* **31,** *1041* (*1948*)).

The inoculation procedure consisted of placing pieces of cud from mature cows into the posterior part of the mouths of the calves. Such inoculations assisted in establishing protozoa in the rumens of calves that were fed milk and hay, or milk with grain and hay. Uninoculated calves failed to establish protozoa in their rumens. There were, however, no differences in weight gains between inoculated and uninoculated calves. The calves fed milk and hay, inoculated or uninoculated, were free of diarrhea. The incidence of diarrhea was over 50 per cent in calves fed milk with hay and grain or milk with a commercial calf starter. When the latter rations were fed, inoculations had no influence on the incidence of diarrhea.

Although protozoa failed to appear in the rumens of uninoculated calves fed milk and hay alone, these calves readily established rumen flora of the types usually present in abundance in mature cows that were fed hay. Since digestive disturbances were not observed in the uninoculated calves fed milk and hay alone, these results appear to indicate that direct rumen inoculation was not so essential in the maintenance of good health as was the early feeding of hay.

The influence of the ratio of grain to hay in the ration of calves between 1 and 4.5 months old on the development of rumen microorganisms was also investigated (*J. Dairy Sci.* **31,** *1051* (*1948*)). Protozoa and organisms associated with hay ingestion generally were absent in rumens of calves on strictly grain rations. Moderate numbers of such organisms were present when hay alone was fed and masses of protozoa and numerous flora of the types associated with hay ingestion were present when only limited amounts of grain were fed with hay. These results again demonstrated the importance of hay or roughage in the ration as a means of early establishment of rumen function in calves.

Rumen inoculations with cud material from cows on pasture were tried in calves that were fed milk and placed on pasture at 4 days of age (*J. Dairy Sci.* **32,** *1025* (*1949*)). There was again no difference in the growth rate of the 6 inoculated and the 6 uninoculated calves used in this study. Four calves, 2 inoculated and 2 uninoculated, had mild diarrhea. The same rumen microorganisms were established on pasture as had been observed to develop when calves were fed hay. Rumen inoculations were again observed to be necessary for early establishment of protozoa.

The influence of ration and early rumen development on the plasma carotenoids, vitamin A, and ascorbic acid were also reported (*J. Dairy Sci.* **31,** *1055* (*1948*)). Rumen inoculations had no effect on blood carotenoids or vitamin A levels but were effective in preventing the drop in blood ascorbic acid which usually occurred between 7 and 14 days of age. This favorable effect from inoculation was noted only in calves fed milk and hay alone. Rumen inoculation had no influence on the blood levels of carotenoids, vitamin A, or ascorbic acid in calves on pasture (*Ibid.* **32,** *1016* (*1949*)). These results again emphasized that hay ingestion by young calves stimulated early development of rumen function and appeared to have a favorable physiologic effect.

These workers concluded that microorganisms characteristic of the rumen contents of older cattle failed to become established in the rumens of young calves mainly because they did not ingest good quality roughage or because they ingested too much grain and not enough roughage. Some evidence was obtained to indicate that rumen inoculation with cud material from mature cows aided in the establishment of desirable microflora and microfauna. However, little direct evidence was presented by these workers to indicate that rumen inoculations had any influence on the health of calves over and above the beneficial effects that resulted from early feeding of good roughage. In all cases the digestive upsets and diarrhea

frequently noted in young calves were apparently as effectively prevented by hay feeding as by rumen inoculation. The only evidence of a difference between inoculated and uninoculated calves receiving good roughage was the observation that the hair coats of the latter were rougher than those of the former. Inoculations likewise did not appear to have any significant influence on the blood levels of the vitamins studied.

Limited observations were reported on calves in farm herds (*J. Am. Vet. Med. Assn.* **114,** *33* (*1949*)). The calves in one of the herds under study were so well separated from the cows that they were not obtaining the usual varieties of rumen microorganisms. These calves were rough in appearance and failed to grow normally. Cud material from a cow in the herd was provided one of the calves when it was 4 months old. The inoculation resulted in improved growth and appearance. These workers believe that rumen inoculations may be particularly useful as a means of insuring early rumen function and healthy calves on farms where the calves are kept away from older stock. They point out that under such conditions calves are deprived of the opportunity of establishing rumen microorganisms through the ingestion of roughages that have become contaminated by contact with cud material from older animals.

The Ohio laboratory has made an important beginning on a problem that should be followed with interest by nutritionists. Further studies of this nature should establish a clearer understanding of the significance of rumen microorganisms in the nutrition of calves. Particularly needed is more precise information on the identity of the organisms that contribute toward healthy rumen function.

URINARY EXCRETION OF PRODUCTS OF NIACIN METABOLISM IN HUMAN SUBJECTS

A variety of end products of niacin metabolism have been isolated from or reported to occur in urine of experimental animals and man fed the vitamin or a precursor (*Nutrition Reviews* **4,** *58* (*1946*); **5,** *233* (*1947*)). The major urinary excretory products in human subjects are N′-methylnicotinamide and N-methyl-6-pyridone-3-carboxylamide. However, little information is as yet available concerning the rates of excretion of these compounds and their quantitative relationships in urine under defined conditions of diet.

W. I. M. Holman and D. J. de Lange (*Nature* **166,** *468* (*1950*)) have determined the quantities of N′-methylnicotinamide and the pyridone excreted in the urine of a normal adult male during three-hour intervals throughout twenty-four-hour periods. The subject consumed his customary diet and attempted to make it reasonably constant during experimental periods. He ate his meals at the same hours of the day. No information is available concerning the niacin or tryptophan content of the diet. After excretion rates had been determined on the diet alone, supplements of niacin, tryptophan, or niacinamide were provided and the quantities of the two excretion products determined on specimens of urine collected during three-hour intervals. A fluorometric procedure was utilized for the determination of N′-methylnicotinamide (J. W. Huff and W. A. Perlzweig, *J. Biol. Chem.* **167,** *157* (*1947*)), while a colorimetric method was used in assaying for pyridone (Holman and de Lange, *Biochem. J.* **45,** *559* (*1949*)).

Excretion of both N′-methylnicotinamide and pyridone remained remarkably constant during a twenty-four-hour period on the diet alone. N′-methylnicotinamide was excreted in quantities of from 0.15 to 0.33 mg. per hour, while the pyridone was excreted at a somewhat greater rate, varying

from 0.48 to 0.67 mg. per hour. There was no evidence that greater quantities of either substance appeared in the urine immediately following consumption of meals. During the day a total of 6.3 mg. of N′-methylnicotinamide and 14.2 mg. of pyridone was excreted. In the three-hour collection periods, amounts of pyridone in the urine exceeded those of N′-methylnicotinamide by factors of from two to four.

One hundred milligrams of niacinamide were ingested by the subject at the beginning of a second experimental day. There was an immediate outpouring of N′-methylnicotinamide, with a rate of excretion of 5.7 mg. per hour during the first three hours. This decreased to 1.9 mg. per hour in the second collection period. After nine hours the rate of excretion was comparable to that on the control day. The pattern of excretion of pyridone was different from that of N′-methylnicotinamide. A maximum rate was reached during the second collection period and greater quantities were excreted during all periods than on the control day. An excess of 26 mg. of N′-methylnicotinamide and 62 mg. of pyridone appeared in the urine as compared to the control. Approximately 85 per cent of the supplement of niacinamide was recovered from the urine in the two excretion products.

One week following tests of excretion with administration of niacinamide, 3 g. of l-tryptophan were given orally to the subject. In this instance excretions of both N′-methylnicotinamide and pyridone were increased. A quantity of 2.8 mg. of the first metabolite and 16.6 mg. of pyridone above control levels was recovered within twenty-four hours. Maximum excretory rates occurred at somewhat later periods than when niacinamide was given.

After another interval of a week, 100 mg. of niacin were administered. Of the amount given, 36 mg. were recovered from the urine within twenty-four hours, 10 mg. of N′-methylnicotinamide and 26 mg. of pyridone. As with urinary excretion following niacinamide, the rate of excretion of pyridone reached its maximum during the second three-hour period and remained elevated throughout the day.

Of particular interest in these observations is the much greater quantity of end products recovered from the urine after administration of niacinamide than with niacin. The authors conclude that provided both compounds are readily absorbed, amidation of niacin is an important factor controlling production of the metabolites which appear in the urine. It will be recalled that other observations also indicate a likelihood that niacin and its amide take somewhat different metabolic pathways (C. L. Hoagland, S. M. Ward, and R. E. Shank, *J. Biol. Chem.* **151,** *369* (*1943*)).

It has been well established that tryptophan is a precursor of niacin (*Nutrition Reviews* **8,** *85* (*1950*)). The data of Holman and de Lange (*loc. cit.*) again give evidence of this in man with increased excretion of N′-methylnicotinamide and pyridone following administration of this amino acid. In extending these observations they also gave 100 g. of an amino acid mixture containing only traces of niacin and tryptophan to 2 subjects. This was not followed by increased excretion of the urinary metabolites. In most instances there was an actual decrease in excretion of N′-methylnicotinamide and pyridone for a period of forty-eight hours. It seemed clear that amino acids other than tryptophan had no important effect in increasing the body pool of niacin.

Procedures are not yet available which make possible an estimate of body stores of niacin or even permit an evaluation of the recent level of intake of the vitamin and its precursors. The combined excretion of N′-methylnicotinamide and pyridone may, with more extended investigation, be shown to provide an approach to the problem of assessment of niacin nutriture.

NOTES

Letter to the Editor

Dear Sir:

In your review on the toxicity of vitamin B_{12} (V. Traina, *Arch. Path.* **49,** *278* (*1950*); *Nutrition Reviews* **8,** *324* (*1950*)) the reviewer mentions the possibility that it may have been not the vitamin, but some foreign substance, which was toxic, and that further study is needed on this point.

We should like respectfully to call your attention to the fact that these studies have been made and published (C. A. Winter and C. W. Mushett, *J. Am. Pharm. Assn. (Sci. Ed.)* **39,** *360* (*1950*)).

This paper demonstrates that crystalline vitamin B_{12} is essentially a nontoxic substance, since doses as high as 1600 mg. per kilogram administered intravenously or intraperitoneally in mice produced no toxic manifestations or deaths. This dose was more than five hundred times as great as that which produced deaths in 100 per cent of the animals as reported by Traina. The authors therefore concluded that the toxic manifestations observed by Traina were probably due either to toxic impurities in the preparation which he used, or to contamination of the sample.

It may be of further interest to note that Traina has also claimed antihistaminic (*Nature* **165,** *439* (*1950*)) and antianaphylactic (*Ibid.* **166,** *78* (*1950*)) activity for vitamin B_{12}. We have been completely unable to confirm these results (*unpublished results*), and two other laboratories have failed to corroborate Traina's observations on the antihistaminic activity of vitamin B_{12} (P. Ducrot, *Compt. rend. Soc. de biol.* **144,** *697* (*1950*); H. M. Sharpe, E. A. Woollett, and W. F. J. Cuthbertson, *Nature* **166,** *651* (*1950*)).

Charles A. Winter
Charles W. Mushett
Merck Institute for Therapeutic Research
Rahway, N. J.

Nitrates in Water

The topic of nitrates in drinking water has aroused considerable interest among food technologists, pediatricians, health officers, and sanitarians.

D. F. Metzler (*Harvard Pub. Health Alumni Bull.* **7,** *8* (*1950*)) has prepared a review of the occurrence of relatively high concentrations of nitrate in water supplies, with a discussion of related risks to health.

Sugar and Dental Caries

A symposium entitled "Sugar and Dental Caries" (*J. Calif. State Dent. Assn.* **26** (*Suppl. to No. 3*) (*1950*)) merits careful evaluation by those in the nutrition field who are interested in the complexities of the dental caries problem. Present knowledge of various facets of this subject has been reviewed with varying skill by seven well-known investigators.

Throughout the symposium there is a wealth of factual material accompanied by detailed discussions of numerous controversial areas. In many ways, the unbiased approach of some of the investigators in their review of the mutual problem and the sharing of their divergent viewpoints on controversial issues in the discussion period adds a scientific wholesomeness to the symposium which is rarely seen in the dental literature.

Three of the sections are particularly well rounded, clearly presented, and discussed in relation to the other sections, namely, "The Role of Sugar in Dental Caries," "Is the Susceptibility to Dental Caries Influenced by Factors Operating During the Period of Tooth Development," and "'Natural' and Refined Sugars, Their Chemistry and Biochemistry." Clearly manifest throughout the symposium is the need in the dental field for a multitude of systematic investigations in which a wide

variety of basic scientific disciplines is employed.

International Food Composition Tables

Some recently published tables prepared by Charlotte Chatfield with the collaboration of various members of the Nutrition Division of FAO ("Food Composition Tables for International Use," *FAO Nutritional Studies, No. 3, Oct. 1949*) represent a considerable sum of work of compilation of original analytical reports, summary of national food composition tables, and studies on digestibility of certain foodstuffs. The data are expressed in terms of calories, protein, and fat contents, and are weighted averages. The method used in arriving at these figures is derived from a previous study ("Energy-yielding Components of Food and Computation of Calorie Values: Report of a Committee on Calorie Conversion Factors and Food Composition Tables," *FAO, Washington, D. C. (1947)*). The first part of the tables gives a clear definition of the meaning of terms which are sometimes confusing to the student of public health nutrition, such as "retail values," "edible portion," "as purchased," "refuse," etc. The tables themselves give food compositions both in terms of retail weight ("as purchased") and of "edible portion" in the material as purchased. Extreme care has been taken with such difficult points as extraction rates, degree of fattening for meats, etc.

While nutritionists operating in a given area might prefer locally established food tables, the FAO food composition tables should prove invaluable for the work of nutritionists in those many regions of the world where no such analytical work has been effected, as well as for any study involving interregional comparisons.

Carrots and Low Oxygen Tension

With the advent of high altitude flying, and particularly of pressurized cabins, interest has been stimulated in the study of possible methods for prolonging consciousness and life under conditions of greatly reduced oxygen tension. Thus the reports that a diet consisting entirely of carrots significantly increased the resistance of rats to hypoxia have been widely quoted (J. A. Campbell, *Quart. J. Exp. Physiol.* **28,** *231 (1938)*; **29,** *259 (1939)*; D. Nelson, S. Goetzl, S. Robins, and A. C. Ivy, *Proc. Soc. Exp. Biol. Med.* **52,** *1 (1943)*). In these experiments all of the animals on the carrot diet lost body weight compared to controls on an ad libitum diet.

C. W. Craven, H. I. Chinn, and R. W. MacVicar (*J. Aviation Med.* **21,** *256 (1950)*) reported a study in which an effort was made to control the possible effect of the reduction in body weight. Three groups of rats were studied. The animals in the first group were given fresh raw carrots and tap water, both ad libitum. The second diet consisted of a commercial dog chow, restricted by paired feeding so that the weight loss in this group of animals was approximately equivalent to that in group I. The third group was given dog chow ad libitum. The survivals in simulated altitudes up to 34,000 feet were studied.

The results demonstrate that the animals on the carrot diet did live longer than those fed the dog chow ad libitum. However, there was not a significant difference between groups I and II, that is, between those fed carrots and those pairs fed dog chow. Thus it appeared clear that food restriction with weight loss led to improved resistance to hypoxia. The reasons for this were not investigated by the authors but they postulated that either a reduction in metabolic rate, and therefore in oxygen requirement, results from the weight loss or perhaps the adaptation syndrome of Selye is brought into play.

NOTICE

Commencing April 1, 1951 the subscription rate to NUTRITION REVIEWS will be $4.00 per year in the United States and Canada and $4.50 in other countries. The increase in subscription rate is due to the increased cost of publishing and distributing this journal.

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